REMARKS

Claim 12 has been canceled without prejudice or disclaimer. Claims 31 and 32 have been added. Claims 31 and 32 are supported by the specification and claims as originally filed, including by original claims 1 and 12.

Claim 1 has been amended to include the limitations found in canceled claim 12. Claim 1 has also been amended to recite that the cells are cultured "in the presence of an effective amount of a selective agent or the absence of an appropriate selective agent." Support for this amendment is found in the original specification, e.g., on page 9, line 27 to page 10, line 2.

Claim 13 has been amended to change its dependency from claim 12 to claim 1 and to further clarify that the "nucleic acid sequence" referred to in this claim is the fungal replication initiating polynucleotide sequence.

Claim 14 has been amended to change its dependency from claim 12 to claim 1.

Claim 17 has been amended to change its dependency from claim 12 to claim 1.

Claim 30 has been amended to correct a minor typographical error.

Accordingly, claims 1-11, 13-22 and 27-32 are now pending and present for examination.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. The Rejection of Claims 1-9, 11-18, 20-21 and 30 under 35 U.S.C. 112

Claims 1-9, 11-18, 20-21 and 30 are rejected under 35 U.S.C. 112, as allegedly not enabled. The Examiner contends in paragraph 8 of the Office Action that the specification allegedly does not sufficiently teach the use of any replication initiating sequence other than the sequences having at least 80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

In order to expedite prosecution, claim 1 has been amended to include the limitations found in dependent claim 12.

The Examiner further contends that the use of "%", as in the percent identity referenced in claims 12-17, is a problem because the Examiner states that sequence identity allegedly has no common meaning within the art. The determination of percent identity between nucleic acid sequences is well-known to those skilled in the art. As disclosed in the specification, the determination of percent identity may be suitably determined by means of computer programs known in the art, such as, the computer program GAP provided in the GCG program package.

See the specification at page 13, lines 5-14. Moreover, claim 1 particularly recites both a specific computer program (namely, the GAP computer program) used to determine percent identity and the specific settings for polynucleotide comparisons, namely, a GAP creation penalty of 5.0 and a GAP extension penalty of 0.3, to be used when determining percent identity using this computer program. Based on the skill of the artisan and the guidance in the specification, and the specific recitation found in the claims, one skilled in the art would unquestionably be able to determine whether or not a sequence has the recited percent identity to SEQ ID NO:1 or SEQ ID NO:2.

For the foregoing reasons, Applicant submits that the claims overcome this rejection under 35 U.S.C. 112. Applicant respectfully requests reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 1-9, 11-18, 20-21 and 30 under 35 U.S.C. 112

Claims 1-9, 11-18, 20-21 and 30 are rejected under 35 U.S.C. 112, as allegedly indefinite. The indefiniteness rejections are addressed as follows:

Claim 1 is rejected as indefinite on the basis that it is allegedly not clear whether the recited method is a method of constructing, selecting or screening a library of polynucleotide sequences. Applicants respectfully submit that the recited method can be used for constructing, selecting or screening a library of polynucleotide sequences. As recited in step (c), after cultivating the cells under a selection pressure, the method involves selecting or screening for one or more transformants expressing a desired characteristic. In this regard, the specification clearly defines what is meant by the phrase "selecting or screening." See the specification on page 23, lines 17-25. Moreover, the claimed method clearly can also involve "constructing" a library as step (a) recites the step of transforming fungal cells with a population of DNA vectors, wherein each vector comprises a fungal selection marker polynucleotide sequence, a fungal replication initiating polynucleotide sequence and a polynucleotide sequence of interest, wherein vectors in the population vary from other vectors in the population by carrying different versions of the polynucleotide sequence of interest. However, in order to shorten the preamble of claim 1, claim 1 has now been amended to recite a method of screening a library of polynucleotide sequences of interest in filamentous fungal cells.

Claim 1 is rejected on the basis that the phrase "selection pressure" is unclear. The Examiner contends that the specification does not have a clear definition for "selection pressure" as "it is not clear whether applicants mean in the presence of an effective amount of an appropriate selective agent, which is selected based on the selection marker." Applicants respectfully disagree that the term "selection pressure". However, as requested by the

Examiner, the claims have been amended to recite that the step (b) involves "cultivating th cells in the presence of an effective amount of a selective agent or the absence of an appropriate selective agent." Support for this amendment is found in the specification on page 9, line 27 to page 10, line 2.

Claim 1 is rejected as indefinite on the basis that the phrase "desired characteristic" is not clear. The Examiner contends that the specification does not provide a definition for the recitation "desired characteristic." The phrase "desired characteristic" is simply used in the specification and claims in its plain and ordinary meaning to refer to a desired property imparted by the nucleotide sequence of interest, such as encoding a polypeptide that has a certain function or activity of interest. In this regard, it is well-established that a patent Applicant need not define each and every term used in the specification and claims, and this is especially true for terms that are used in their plain and ordinary meaning and in a manner that would clearly be understood by one skilled in the art. However, notwithstanding the above, a definition and examples of desired characteristics are indeed provided in the specification (see the specification on page 23, line 12 to page 24, line 17), as follows:

> The term *"selecting or screening" for one or more transformants expressing a desired characteristic"* as used in step c) is intended to indicate that the screening or selecting is performed so as to identify transformants containing a modified polynucleotide sequence of interest (which has been generated on the basis of the polynucleotide sequences of interest during the cultivation step b) which has the desired activity or function and optionally further desired characteristics as exemplified below. Thus, if the polynucleotide sequence of interest encodes a polypeptide with a certain activity or function the selection will only allow the transformants expressing a polypeptide with the desired activity or function, to grow. Thus, if the polynucleotide sequence of interest encodes a polypeptide with a certain activity or function the screening will be performed to identify transformants expressing a polypeptide with the desired activity or function. For instance, if the polynucleotide sequence of interest encodes an enzyme, such as a lipase, the selection or screening step c) will be performed to identify transformants expressing lipase activity. If it is desired that the lipase to be identified as a specific characteristic, such as a high thermostability, the screening is to be performed under conditions (typically temperatures) at which lipases with the desired high thermostability can be identified.
>
> Analogously, if the polynucleotide sequence of interest is a control sequence such as a promoter sequence the selection or screening step c) is performed under condition in which promoter activity can be assessed. Typically, in the library the promoter polynucleotide sequences of interest are operably linked to a

> second sequence to be transcribed (e.g. a polypeptide encoding sequence) so that the promoter activity can be assayed with reference to the transcription of said second sequence.

(Emphasis added.) The term "desired characteristic" is therefore both clearly defined and clearly exemplified in the specification.

Claim 1 is rejected as allegedly incomplete for omitting an essential step. The Examiner contends that the claims do not recite *how* to select or screen for one or more transformants expressing a desired characteristic since it is allegedly not known what "desired characteristic" is being sought. Foremost, the present invention is clearly not restricted to identifying a particular "desired characteristic", as the methods of the present invention can be used to identify any number of desired characteristics. Furthermore, as previously discussed, the term "desired characteristic" is not unclear, but is instead both well-defined in the specification and would be well-understood to refer to a property imparted by a polynucleotide sequence of interest (e.g., encoding a polypeptide having a desired function or activity). Claim 1 recites a screening method and incluces the steps of transforming fungal cells with library comprising polynucleotide sequences of interest (and a fungal selection marker and fungal replication initiating sequence), cultivating the cells, and selecting or screening for one or more transformants expressing a desired characteristic. All of the essential steps are present as these are the steps which an artisan would carry out to practice the claimed screening method. Applicants therefore respectfully submit that an essential step is not missing from the claims.

Claim 4 is rejected on the basis that the phrase "a portion thereof" is unclear. The Examiner contends that it is not clear whether the portion of the enzyme or receptor has any specific function or structure. The phrase "or portion thereof", simply refers to that which is less than the entire receptor or antibody. The phrase "or portion thereof" is therefore not unclear. Moreover, the Examiner is reminded that Applicants are not claiming the nucleic acid sequence of a portion of a specific receptor or a specific antibody, but rather the claims are directed to methods for selecting or screening a library of polynucleotide sequences, and claim 4 is directed (*inter alia*) to a method of screening nucleic acid sequences encoding a portion of a receptor or a portion of an antibody of interest. In this regard, the scientific literature is replete with publications addressing the importance of parts of antibodies or parts of receptor, and disclosing the nucleic acid sequences encoding these portions, such that that an artisan who has interest in screening or selecting a library of polynucleotide sequences which encode only portions of antibodies or portions of a receptors, would plainly be able to carry out this task using the claimed screening method. Thus, within the context of the present invention, it is

clear that the phrase "a portion thereof" refers to less than all of the entire receptor or entire antibody.

Claims 12-16 are rejected as indefinite on the basis that the recitation "% identity" is unclear. As previously discussed in response to the enablement rejection, the determination of percent identity between nucleic acid sequences is well-known and a routine practice to those skilled in the art. Moreover, claim 1, specifically recites not only a specific computer program an artisan can use to determine percent identity, but also the specific settings for polynucleotide comparisons that should be employed when using this program, namely, a GAP creation penalty of 5.0 and a GAP extension penalty of 0.3. Applicant therefore respectfully submits that the recitation "% identity" is plainly not indefinite.

Claim 18 is rejected on the basis that the phrase "wherein the modification of patent polynucleotide sequence" has insufficient basis. Claim 18 is also rejected on the basis that the term "preferably" is indefinite. In the Amendment filed on September 8, 2001, Applicants addressed these alleged defects of claim 18 by amending claim 18, as follows:

> 18. (Amended.) The method of claim 2, wherein the [modification of the parent] polynucleotide sequence <u>of interest</u> [is performed] <u>was created</u> by mutagenesis, [preferably] <u>by</u> random mutagenesis, by use of a physical or chemical mutagenizing agent, <u>by</u> use of a doped oligonucleotide, <u>by</u> DNA shuffling, [or] by subjecting the nucleic acid sequence to PCR generated mutagenesis, or <u>by</u> use of any combination thereof.

Applicants respectfully submit that the indefiniteness rejections of claim 18 were successfully overcome in the Amendment filed on September 8. Applicants respectfully request reconsideration and withdrawal of the rejection.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 1-9, 11 and 18-21 under 35 U.S.C. 103

Claims 1-9, 11, 18-21 are rejected under 35 U.S.C. 103 as being unpatentable in view of Christensen and Dalboge et al.

In order to expedite prosecution, claim 1 has been amended to include the recitations found in claim 12, which is indicated to be free of the prior art.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 103. Applicants respectfully request reconsideration and withdrawal of the rejection.

VII. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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Attorney Docket No.: 5579.210-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jesper Vind Confirmation No: 1334

Serial No.: 09/426,038 Group Art Unit: 1627

Filed: October 25, 1999 Examiner: P. Ponnaluri

For: Methods Of Constructing And Screening A DNA Library Of Interest In Filamentous Fungal Cells

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

IN THE CLAIMS:

The claims have been amended as follows:

1. (Twice Amended.) A method of [constructing and selecting or] screening a library of polynucleotide sequences of interest in filamentous fungal cells, wherein the method comprises:

(a) transforming the fungal cells with a population of DNA vectors, wherein each vector comprises:

(iii) a fungal selection marker polynucleotide sequence and a fungal replication initiating polynucleotide sequence, wherein the marker and the replication initiating sequence do not vary within the population; and wherein the replication initiating sequence is a nucleic acid sequence selected from the group consisting of: (1) a replication initiating sequence having at least 80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, as determined using the GAP computer program with a GAP creation penalty of 5.0 and GAP extension penalty of 0.3, and is capable of initiating replication; and (2) replication initiating sequence which hybridises under low stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, or (ii) the respective complementary strands, wherein the low stringency conditions are defined by prehybridization and hybridization at 42°C in 5x SSPE, 0.3% SDS, 200 mg/ml sheared and denatured salmon sperm DNA, and 25% formamide, and wash conditions are defined at 50°C for 30 minutes in 2x

SSC, 0.2% SDS; and

(iv) a polynucleotide sequence of interest, wherein there are vectors in the population that vary from other vectors in the population by carrying different versions of the polynucleotide sequence of interest;

(b) cultivating the cells [under a selection pressure] in the presence of an effective amount of a selective agent or the absence of an appropriate selective agent;

(c) selecting or screening for one or more transformants expressing a desired characteristic; and

(d) isolating the transformant(s) of interest.

2. (Unchanged.) The method according to claim 1, wherein the library of polynucleotide sequences of interest is prepared by random mutagenesis or naturally occurring allelic variations of at least one parent polynucleotide sequence having or encoding a biological activity or function of interest.

3. (Unchanged) The method of claim 1, wherein the polynucleotide sequence further comprises a control sequences.

4. (Unchanged) The method according to claim 1, wherein the polynucleotide sequence of interest encodes a hormone, an enzyme, a receptor or a portion thereof, an antibody or a portion thereof, or a reporter, or a regulatory protein.

5. (Unchanged.) The method of claim 4, wherein the enzyme is an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, or a ligase.

6. (Unchanged.) The method according to claim 4, wherein the enzyme is an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, a proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

7. (Unchanged) The method according to claim 3, wherein the control sequence is an enhancer sequence, a leader sequence, a polyadenylation sequence, a propeptide sequence, a promoter, a replication initiation sequence, a signal sequence, a transcriptional terminator or a translational terminator.

8. (Unchanged.) The method of claim 7, wherein the promoter is derived from the gene encoding *Aspergillus oryzae* TAKA amylase, NA2-tpi and *Aspergillus niger* or *Aspergillus awamori* glucoamylase.

9. (Unchanged.) The method according to claim 1, wherein the selection marker polynucleotide sequence is selected from the group of genes which encode a product which is responsible for one of the following: resistance to biocide or viral toxicity, resistance to heavy metal toxicity, prototrophy to auxotrophs.

11. (Unchanged.) The method of claim 9, wherein the selection marker polynucleotide sequence is a gene selected from the group consisting of *argB* (ornithine carbamoyltransferase), *amdS* (acetamidase), *bar* (phos-hinothricin acetyltransferase), *hemA* (5-aminolevulinate synthase), *hemB* (porphobilinogen synthase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *prn* (proline permease), *pyrG* (orotidine-5'-phosphate decarboxylase), *pyroA*, *riboB*, *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase).

13. (Twice Amended) The method of claim [12] 1, wherein the [nucleic acid sequence] replication initiating polynucleotide sequence has at least 80% identity with the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, as determined using the GAP computer program with a GAP creation penalty of 5.0 and GAP extension penalty of 0.3.

14. (Twice Amended.) The method of claim [12] 1, wherein the replication initiating polynucleotide sequence is obtained from a filamentous fungal cell.

15. (Unchanged.) The method of claim 14, wherein the filamentous fungal cell is a strain of *Aspergillus*.

16. (Unchanged.) The method of claim 15, wher in the strain of *Aspergillus* is obtained from a strain of *A. nidulans*.

17. (Twice amended.) The method of claim [12] 1, wherein the replication initiating polynucleotide sequence has the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

18. (Unchanged.) The method of claim 2, wherein the polynucleotide sequence of interest was created by mutagenesis, by random mutagenesis, by use of a physical or chemical mutagenizing agent, by use of a doped oligonucleotide, by DNA shuffling, by subjecting the nucleic acid sequence to PCR generated mutagenesis, or by use of any combination thereof.

19. (Unchanged.) The method of claim 18, wherein the polynucleotide sequences of interest are obtained by in vivo recombination between two or more homologous nucleic acid sequences encoding a polypeptide or a regulatory sequence, or any combination of both, comprising:
    (a) identifying at least one conserved region between the polynucleotide sequences of interest;
    (b) generating fragments of each of the polynucleotide sequences of interest, wherein said fragments comprise the conserved region(s) of (a); and
    (c) recombining the fragments of (b) by using the conserved region(s) as (a) homologous linking point(s).

20. (Unchanged.) The method according to claim 1, wherein the filamentous fungal cell transformed with the population of DNA vectors is a cell of a strain of *Acremonium*, *Aspergillus*, *Coprinus*, *Fusarium*, *Humicola*, *Mucor*, *Myceliopthora*, *Neurospora*, *Penicillium*, *Thielavia*, *Tolypocladium* or *Trichoderma*.

21. (Unchanged.) The method according to claim 20, wherein the cell is an *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus nidulans*, *Coprinus cinereus*, *Fusarium oxysporum*, or *Trichoderma reesei* cell.

30.(Amended.) The method of claim 1, wher in the polynucleotide sequence of interest is a control sequence[s].